University of Alberta

Amygdala Lesions Do Not Impair Shock-Probe Avoidance Retention Performance

by

Hugo Lehmann

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

Department of Psychology

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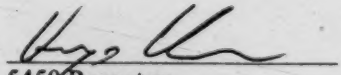

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The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *Amygdala Lesions Do Not Impair Shock-Probe Avoidance Retention Performance* submitted by *Hugo Lehmann* in partial fulfillment of the requirements for the degree of Master of Science.

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Abstract

Amygdala Lesions Do Not Impair

Shock-Probe Avoidance Retention Performance

Hugo Lehmann

The present experiment used the shock-probe paradigm, a procedure usually used to assess anxiolytic processes, to assess memory in amygdala-lesioned rats. Rats were placed in a chamber that contained a probe protruding from 1 of 4 walls and were kept in the chamber for 15 min after they contacted the probe. For half the rats, the probe was electrified (2 mA). Four days later, sham or neurotoxic amygdala lesions were induced. Retention performance was assessed 8 days later by measuring the latency to contact the probe and the number of contact-induced shocks. The results indicated that, although shock-naïve amygdala-lesioned rats were impaired on the second shock-probe test, shock-experienced amygdala-lesioned rats were not. These data indicate that the memory of a shock experience, as indexed with a shock probe avoidance response, is spared in rats with large amygdala lesions.

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Extensive evidence has indicated that amygdala lesions impair the expression of aversive or fearful memories. For example, in Pavlovian fear conditioning, amygdala lesions impair freezing to a stimulus, such as a tone or light, that was previously paired with shock (Helmstetter, 1992; LeDoux, Cicchetti, Xagoraris, & Romanski, 1990; Phillips & LeDoux, 1992). Amygdala lesions also impair instrumental fear conditioning, where an animal is required to make or inhibit a response in order to avoid a shock (Bucherelli, Tassoni, & Bures, 1992; Dunn & Everitt, 1988; Liang et al., 1982; Parent, Quirarte, Cahill, & McGaugh, 1995; Werka, Skar, & Ursin, 1978; Werka & Zeilinsky, 1998). However, it is not always clear whether these lesions affect memory or performance because amygdala lesions can modify non-mnemonic processes that could influence retention performance. For example, amygdala lesions can increase activity levels (Burns, Annett, Kelley, Everitt, & Robbins, 1996; Lorenzini, Bucherelli, Giachetti, Mugnai, & Tassoni, 1991; Parent, Avila, & McGaugh 1995; Parent, Tomaz, & McGaugh, 1992; Vazdarjanova & McGaugh, 1998). Further, amygdala lesions may influence unlearned fear (Bellgowan & Helmstetter, 1996). For example, when presented with innately aversive stimuli, such as predators or novel situations, amygdala-lesioned rats avoid these stimuli less and freeze less than do control rats (Blanchard & Blanchard, 1972; Burns et al., 1996; Dunn & Everitt, 1988; Kemble, Blanchard, & Blanchard, 1990; Kesner, Berman, & Tardif, 1992).

Clearly, these effects of amygdala lesions on unconditioned fear or activity levels could interfere with the accurate assessment of memory. In the inhibitory avoidance paradigm, for example, memory is typically assessed by measuring the

latency to enter an area in which an animal previously received a shock. When retention is tested, rats with amygdala lesions are impaired in this task in that they enter the shock area more quickly than do sham rats (Bucherelli et al., 1992; Dunn & Everitt, 1988; Liang et al., 1982; Parent, Quirarte, et al., 1995). Although one possible interpretation of this deficit is that the amygdala is critically involved in the storage of the memory of that fearful experience, an alternative possibility is that lesion-induced hyperactivity or decreased fear of a shock (that might otherwise be remembered) contributes to the shorter retention latencies and consequently leads to, at the very least, an underestimate of memory.

If the amygdala is involved in the retention of learned avoidance responses, then amygdala lesions should impair expression of avoidance memory in a variety of situations. To test this, we used the shock-probe paradigm to assess avoidance memory in amygdala-lesioned rats. In the shock-probe test, rats are placed in a chamber that contains an electrified probe, and the number of contact-induced shocks are measured (Treit, Pesold, & Rotzinger, 1993). Shock-probe avoidance has an advantage over standard inhibitory avoidance paradigms in that shock-probe is not as dependent on normal activity levels; rats can move freely and still avoid the probe. Shock-probe avoidance is typically assessed in a rat once and used as a measure of anxiety (Treit et al., 1993). To examine memory, we assessed shock-probe avoidance twice: before and after the induction of large amygdala lesions. Furthermore, we assessed shock-probe avoidance in rats that were and were not given shock experience before the induction of the lesions. The results indicate that amygdala lesions impair shock-probe performance in shock-naïve rats; however, the amygdala

lesions do not impair the expression of memory of the shock in shock-experienced rats. These findings indicate that, although the amygdala may be involved in the acquisition or consolidation of shock-probe avoidance, it is not critically involved in the retention of this, and perhaps other, learned fear associations.

Method

All procedures were approved by the University of Alberta Biosciences Animal Policy and Welfare Committee and carried out in accordance with the guidelines of the Canadian Council on Animal Care (CCAC).

Subjects

Male Sprague Dawley rats (Ellerslie Laboratories, Edmonton, Alberta, Canada; 250-300 g) were housed individually and kept on a 12-hr light-dark cycle (lights on at 0700). They were provided with food and water ad libitum, and allowed to acclimate to vivarium conditions for 1 week before the experiment.

Surgical Procedures

Rats were given atropine sulfate (0.2 cc, 0.5 mg/ml, ip; Ormond Veterinary Supply Ltd., Anchester, Ontario, Canada) and anesthetized with sodium pentobarbital (50 mg/kg, ip; Abbott Laboratories Ltd., Toronto, Ontario). Supplemental doses of sodium pentobarbital (25 mg/kg, ip) were given as needed to maintain anesthesia. Once anesthetized, the rats were hydrated with 0.9% saline (wt/vol; 3.0 cc, sc) and administered antibiotics to reduce the probability of infection (0.05 cc, im; Penicillin G Procaine 300,000 IU/ml; Rhône Mérieux Canada, Victoriaville, Quebec, Canada). The rats were then placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA), and a midline scalp incision was made to expose the top of the skull. Two holes were drilled into the skull (2.3 mm posterior from bregma and 5.0 mm lateral to the midline in both hemispheres; Paxinos & Watson, 1997) and an injection needle (30 gauge, 12.5 mm) was lowered to the area immediately dorsal to the amygdala (-6.7 mm from dura). The injection needle was attached to a 10 µl Hamilton syringe with

polyethylene tubing (PE-50), and the bilateral lesions were induced sequentially by infusing *N*-methyl-D-aspartic acid (NMDA; 0.8 µl; 10 µg/µl over 4 min; Sigma Chemical, St. Louis, MO) with a micro-infusion pump (Harvard Apparatus, St-Laurent, Quebec). The injection needle was left in place for 4 min after the injection to maximize diffusion. The same procedure was used for the sham surgeries, with the exception that the injection needle was not lowered into the brain. Each rat received another injection of 0.9% saline (3.0 cc, sc) at the end of surgery.

Behavioral Procedures

For the acquisition session, each rat was placed into the shock-probe apparatus, which consisted of a Plexiglas chamber (40 cm long × 30 cm wide × 40 high cm) with a wire-wrapped Plexiglas probe (6.0 × 0.5 × 0.5 cm) protruding from the center of one of the walls, 2 cm above the floor (Treit et al., 1993). For half the rats, the probe was constantly electrified (2 mA; shock-experienced) and for the other half it was not (shock-naïve). The rats were removed from the apparatus 15-min after the first contact with the probe. Latency to the first contact-induced shock and the total number of contact-induced shocks were measured in each rat exposed to the electrified probe. The amount of time the rats spent immobile (e.g., standing still or lying on the chamber floor) was measured. In addition, the rats' behavioral reaction to each shock was scored according to a 4-point scale that ranges from a score of 1 for a flinch involving head or forepaw to a score of 4 for a whole-body flinch and jump (all four feet in the air) followed by running to the opposite end of the chamber (Treit et al., 1993). Rats were then matched on the number of contact-induced shocks and shock reactivity and assigned to receive either sham or amygdala lesions 4 days

after the shock-probe acquisition session. Amygdala lesions were also induced in half of the shock-naïve rats.

Eight days after surgery, retention performance was assessed in a second 15-min shock-probe session. The procedure was the same as for the acquisition session, with the exception that the probe was electrified for all rats. For both the acquisition and retention sessions, the behavior of each rat was videotaped and scored by an observer unaware of the rat's surgical status and prior shock-probe history.

Histology

After the completion of the behavioral testing, each rat was overdosed with chloral hydrate (1 cc; 800 mg/ml, ip) and perfused intracardially with 0.9 % phosphate-buffered (PB) saline (wt/vol) followed by 10% (vol/vol) PB-formalin. The brains were stored in a 10% PB formalin-30% (wt/vol) sucrose solution for at least 48 hr and then sectioned (40 μm), mounted on gelatin-coated slides, and stained with thionin. The stained sections were examined through a light microscope (Ernst Leitz Ltd., Midland, Ontario) by an observer who was unaware of the behavioral results. The behavioral results of rats with unilateral lesions or misplaced lesions (i.e., outside the amygdala) were excluded from the statistical analyses. The amount of damage to the central nucleus and basolateral complex (lateral, basolateral, and accessory basal nuclei) of the amygdala in each hemisphere was estimated by using sections from the rostral, middle, and caudal amygdala (-1.8, -2.8, and -3.8 mm relative to bregma; Paxinos & Watson, 1997). The three estimates from each hemisphere were averaged to compute an estimate of total lesion size for each rat.

Statistical Analysis

The number of contact-induced shocks, shock reactivity, and time spent immobile on the retention test were analyzed with between-groups, 2×2 analyses of variance (ANOVAs) with lesion (sham vs. amygdala) and treatment (shock-experienced vs. shock-naïve) as factors, followed by Bonferroni pairwise comparisons where appropriate. The latencies for the first contact-induced shock on the retention test were not normally distributed and were therefore analyzed with the nonparametric Kruskal-Wallis one-way analysis of variance followed by Mann Whitney U-tests for posthoc comparisons. The number of contact-induced shocks for the shock-experienced rats were compared for the acquisition session and the retention test with a mixed design, 2 × 2 ANOVA, with lesion as the between-groups factor and test session (acquisition vs. retention) as the within-groups factors. This was followed by Bonferroni pairwise comparisons when applicable.

Results

Memory Measures

Regardless of whether they had received sham or amygdala lesions, the shock-probe avoidance of rats given preoperative shock experience was better than that of shock-naïve rats (see Figure 1). On the postoperative retention test, rats given preoperative shock experience had longer retention latencies, $U = 121.5$, $p < .01$ and fewer contact-induced shocks, $F(1,43) = 51.179$, $p < .001$ than rats that were not given shock experience. More specifically, shock-experienced sham-lesioned rats had longer retention latencies ($p < .001$) and fewer contact-induced shocks ($p < .001$) than did shock-naïve sham-lesioned rats. Similarly, compared to shock-naïve amygdala-lesioned rats, shock-experienced amygdala-lesioned rats had longer retention latencies, $U = 29.0$, $p < .05$ and fewer contact-induced shocks ($p < .001$), suggesting that amygdala-lesioned rats remembered their previous shock experience. Although amygdala lesions increased the overall number of contact-induced shocks on the retention test, $F(1,43) = 17.802$, $p < .001$, pairwise comparisons indicated that this effect was restricted to the shock-naïve amygdala-lesioned rats ($p < .001$). Shock-experienced lesioned rats were not impaired on the retention test. Neither the retention latencies, $U = 102.00$, $p = .072$, nor the number of contact-induced shocks ($p = .297$) significantly differed between shock-experienced sham- and amygdala-lesioned rats. Memory of the shock-probe experience was also indicated when pre- and post-operative shock-probe avoidance was compared, $F(1,22) = 51.433$, $p < .001$ (see Figure 2). Both sham- and amygdala-lesioned rats received fewer contact-

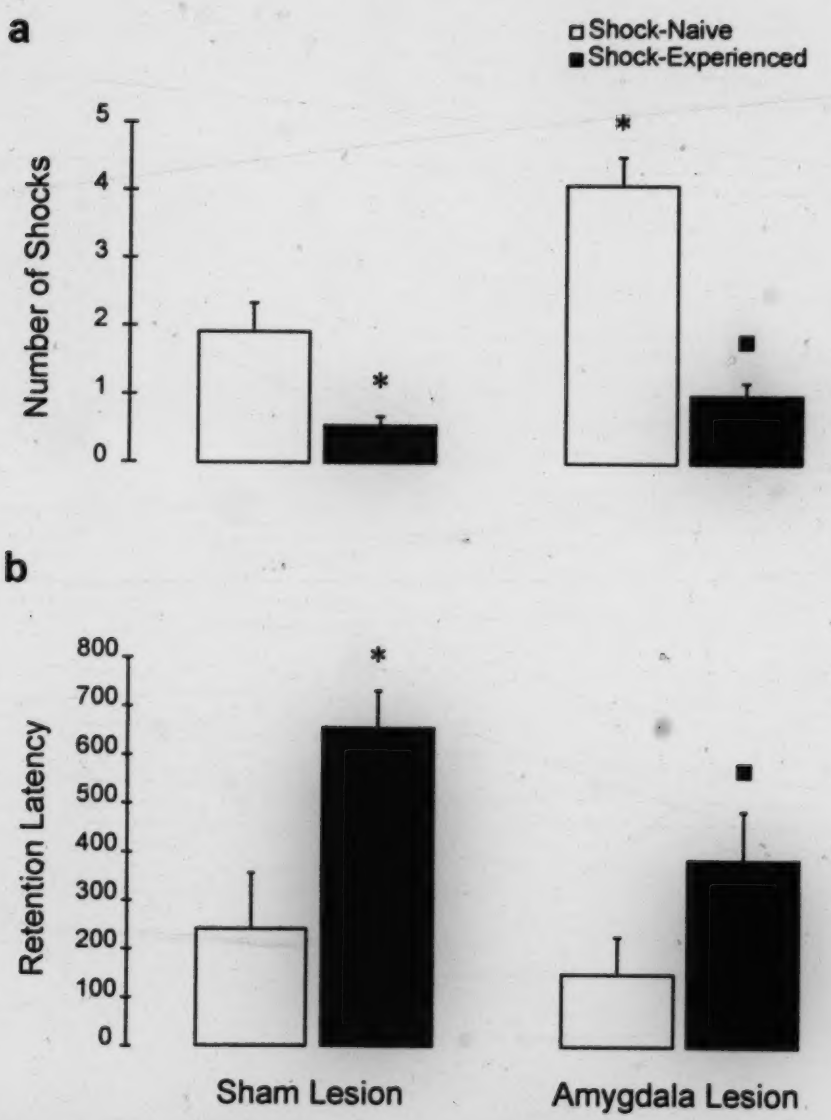

Figure 1. Mean (± *SEM*) (a) number of contact-induced shocks and (b) latency to the first contact-induced shock (retention latency) observed in sham- and amygdala-lesioned rats that were (shock-experienced) and were not (shock-naïve) given preoperative shock-probe experience (* $p < .05$ versus sham-lesioned shock-naïve; ■ $p < .05$ versus amygdala lesion shock-naïve; n = 11-13 per group).

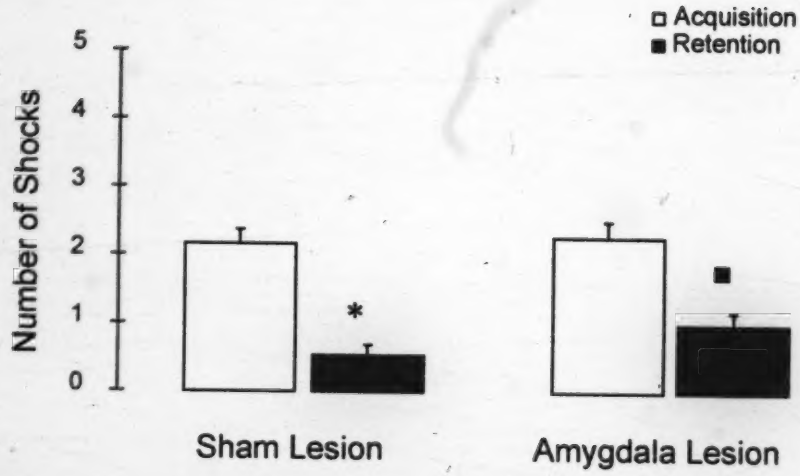

Figure 2. Memory of the preoperative shock-probe experience as assessed by comparing the mean (± *SEM*) number of contact-induced shocks on the acquisition and retention sessions. The retention data are the same as those illustrated in Figure 1a (* $p < .001$ versus sham-lesioned acquisition; ■ $p < .001$ versus amygdala-lesioned acquisition).

induced shocks on the retention test than during the acquisition session ($p < .001$ for both comparisons).

Histological Results

Figure 3 illustrates the smallest and largest amygdala lesions observed in the 22 rats that were included in the behavioral analyses. Figures 4 and 5 show photomicrographs of a representative amygdala lesion and a corresponding photomicrograph from a control rat at two different magnifications. The lesions encompassed the central nucleus (shock-naïve rats: $M = 73.57\% \pm 10.48$; shock-experienced: $M = 82.75\% \pm 4.94$) and basolateral complex (shock-naïve rats: $M = 64.08\% \pm 7.84$; shock-experienced: $M = 79.45\% \pm 3.90$). As can be seen, many of the lesions were large, extended beyond the amygdala, and affected the substantia innominata (n = 20), nucleus basalis (n = 18), the caudate nucleus (n = 22), the globus pallidus (n = 21), ansa lenticularis (n = 16), dorsal or ventral endopiriform nucleus (n = 20), or cortex ventral or lateral to the amygdala (n = 20) in at least one hemisphere.

Although lesion size did vary, the degree of avoidance observed in lesioned rats was not related to these variations. There was no relationship between lesion size and retention latency (shock-naïve: $r = -.084$, $p > .05$; shock-experienced: $r = -.169$, $p > .05$; all lesioned combined: $r = .02$, $p > .05$) or the number of contact-induced shocks on the retention test (shock-naïve: $r = .146$, $p > .05$; shock-experienced: $r = .096$, $p > .05$; all lesioned combined: $r = -.158$, $p > .05$).

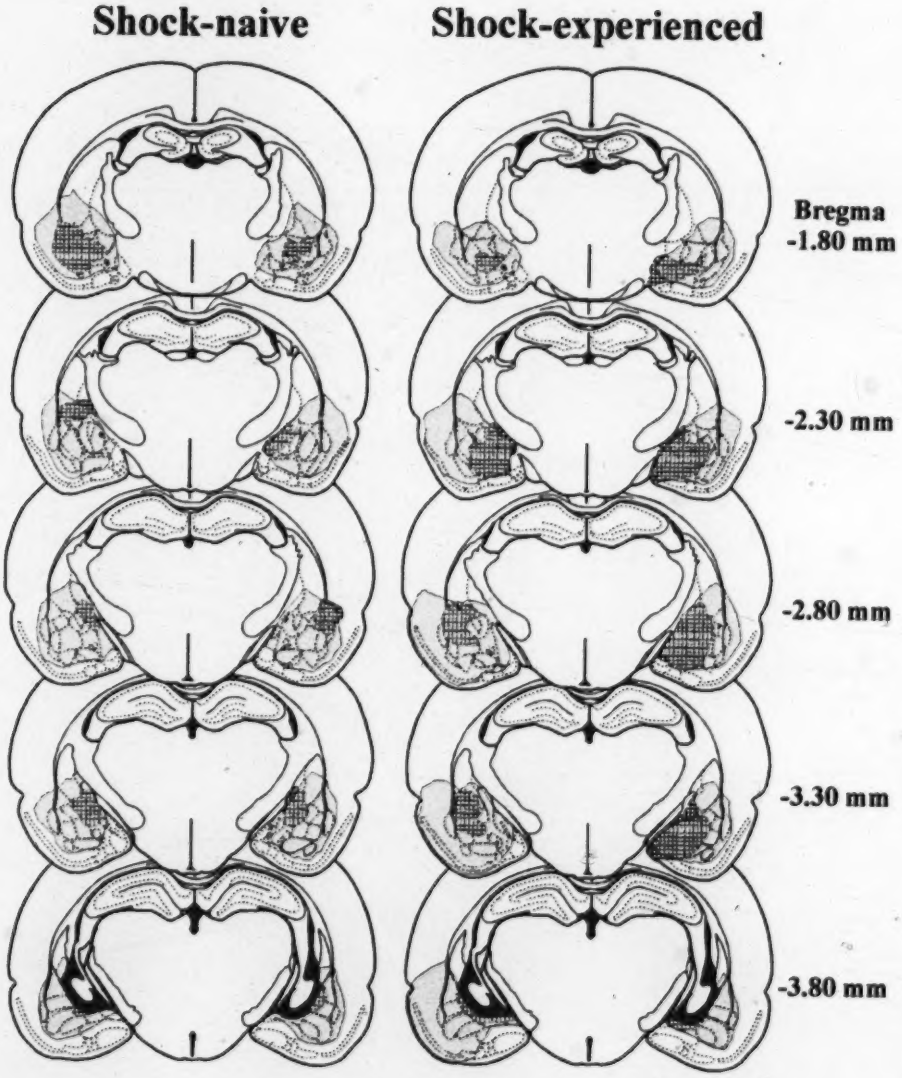

Figure 3. Illustrations of the smallest (crosshatched) and largest (gray shading) lesions observed bilaterally at each 0.5 mm through the rostral and caudal extent of the amygdala for shock-naïve and shock-experienced rats. Adapted from Paxinos and Watson (1997).

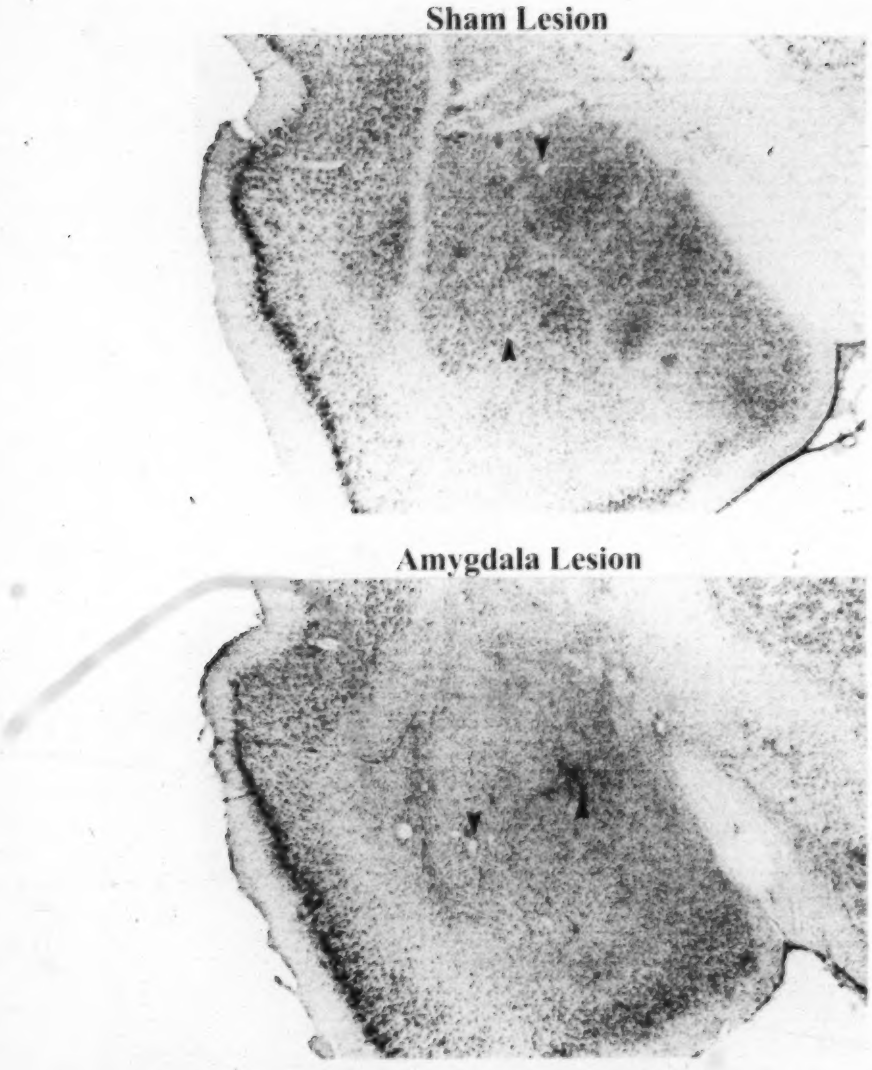

Figure 4. Photomicrographs of a representative amygdala lesion and a corresponding photomicrograph from a sham rat (25 x magnification). The sections correspond to the middle of the amygdala in the rostral-caudal plane (-2.8 mm relative to bregma; Paxinos and Watson, 1997). Arrowheads identify corresponding landmarks from Figure 5.

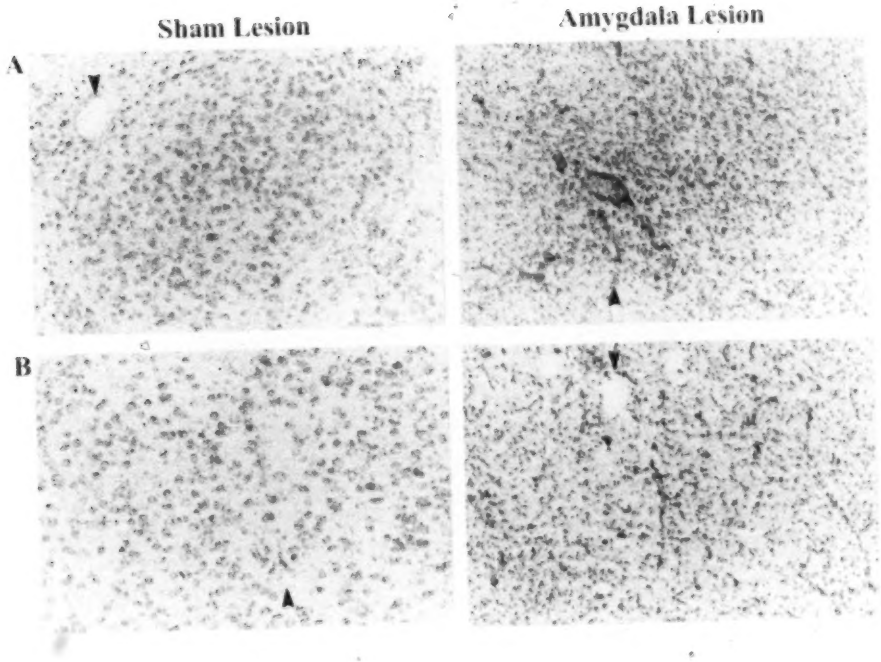

Figure 5. Higher magnification (100 x) photomicrographs of (A) the central nucleus and (B) the basolateral complex of the same sham- (left panel) and amygdala-lesioned rats (right panel) shown in Figure 4. Arrowheads identify corresponding landmarks from Figure 4.

Nonmemory Measures

The effects of manipulating shock-probe experience and amygdala lesions on retention performance were not paralleled by the effects of these manipulations on shock reactivity or general activity. Although there was a significant effect of shock experience on shock reactivity on the retention test, $F(1,32)= 6.276$, $p < .05$, there was no significant effect of lesion, $F(1,32)= 2.610$, $p > .05$; nor was there a significant interaction between shock experience and lesion $F(1, 32)= .095$, $p > .05$. Thus, all rats with previous shock experience (sham and lesioned combined) were more reactive to the shock than were shock-naïve rats (sham-naive: $M = 1.91 \pm 0.32$, sham-experienced: $M = 2.57 \pm 0.20$, lesion-naïve: $M = 1.59 \pm 0.13$; lesion-experienced: $M = 2.11 \pm 0.26$).

Although there was a tendency for shock experience and amygdala lesions to affect time spent immobile on the retention test, a two-way ANOVA (lesion x experience) revealed no significant effect of shock experience, $F(1,43)= 3.318$, $p = .076$, no significant effect of the lesion, $F(1,43)= 2.978$, $p = .092$, and no significant interaction between shock experience and lesion, $F(1,43) = 2.252$, $p > .05$. Mean immobility times (in seconds) were: sham-naive: 124,67 ±44.17; sham-experienced: 39.92 ±17.25; lesion-naïve: 42.36 ±11.52; lesion-experienced: 34.18 ±8.85.

Discussion

Combined, the findings of the present experiment indicate that rats given a shock-experience before the induction of amygdala lesions are able to express memory of the experience on a postoperative retention test given 8 days after the induction of the lesions. Whether the retention performance of shock-experienced amygdala-lesioned rats was compared to that of shock-experienced sham rats, lesioned shock-naïve rats, or with their own pre-operative performance, the results unequivocally demonstrated that the memory of a shock experience is spared in rats with amygdala lesions. Specifically, on the postoperative retention test, the latency to contact the electrified probe and the number of contact-induced shocks did not differ between shock-experienced sham- and amygdala-lesioned rats. Also, compared with their respective shock-naïve controls, both shock-experienced sham- and amygdala-lesioned rats had longer retention latencies and received fewer contact-induced shocks. Finally, compared to their performance on the preoperative shock-probe acquisition session, both shock-experienced sham- and amygdala-lesioned rats received fewer contact-induced shocks on the post-lesion retention test.

These results add to the growing body of evidence indicating that amygdala-lesioned rats retain some memory of a shock experience. For example, several studies have shown that lesioned animals given preoperative footshock training have better retention performance than do lesioned animals not given preoperative training (Parent, Avila, et al., 1995; Parent, Quirarte, et al., 1995; Parent, West, & McGaugh, 1994). However, in all of these studies, a deficit in the avoidance response remained; that is, the lesioned animals are impaired relative to sham controls. The present

findings extend these previous findings by demonstrating that amygdala-lesioned rats are not significantly impaired in a shock-probe avoidance task when compared with control rats. This sparing of memory was observed on two different measures: the number of shocks taken and latency to approach the probe.

For a number of reasons, it is not likely that the memory observed in shock-experienced lesioned rats was due to incomplete destruction of the amygdala. The results of many studies have shown that the concentration and volume of NMDA used in this study is more than sufficient for inducing large lesions (Bermudez-Rattoni, Introini-Collison, Coleman-Mesches, & McGaugh, 1997; Nerad, Ramirez-Amaya, Ormsby, Bermudez-Rattoni, 1996; Parent et al., 1992, 1994; Parent, Quirarte, et al., 1995). We have shown previously that amygdala lesions induced in this manner create robust deficits (Parent et al., 1992, 1994; Parent, Quirarte, et al., 1995). Further, the present study's finding that the lesions produced impaired shock-probe avoidance in shock-naïve rats serves as a positive control for the effectiveness of the lesions. The finding that there was no relationship between lesion size and retention performance also fails to support the possibility that spared tissue mediated the memory that was observed. In most cases, the lesions were large and often encroached surrounding structures and affected both the central and basolateral subregions of the amygdala bilaterally. Interestingly, shock-experienced rats with the best retention performance (no shocks experienced; 15-min retention latency) had some of the largest lesions observed, encompassing the entire rostral-caudal extent of the amygdala and affecting both the central and basolateral region bilaterally. It is important to note, however, that the basolateral region has a higher concentration of

NMDA receptors than the central nucleus (Monaghan & Cotman, 1985). Although previous researchers have provided behavioral and histological evidence indicating that NMDA can effectively lesion the central nucleus (Maisonnette, Kawasaki, Coimbra, & Brandao, 1996; Manning & Mayer, 1995), it is possible that the lesions spared some cells in the central nucleus and that these spared cells mediated the expression of memory. However, the basolateral region has been implicated as the critical region in Pavlovian fear conditioning (Davis, Rainnie, & Cassell, 1994; LeDoux, 1995), and posttraining basolateral complex lesions, but not central nucleus lesions, impair inhibitory avoidance (Parent, Avila, et al., 1995; Parent & McGaugh, 1994; Roozendaal, Koolhaas, & Bohus, 1993). Nonetheless, it will be important in the future to examine the effects on shock-probe avoidance of lesions that are induced using other methods and of discrete lesions of the central nucleus or basolateral area.

It is improbable that the spared memory observed in lesioned rats is due to overtraining produced by the preoperative shock experience. In the present experiment, shock-experienced rats received an average of two shocks before the induction of the lesion. The number of shocks is comparable to, and in many cases less than, the number used in other studies of the amygdala in memory (Bermudez-Rattoni et al., 1997; Campeau & Davis, 1995; Hitchcock & Davis, 1986; Lee, Walker, & Davis, 1996; Maren, Aharanov, & Fanselow, 1996; Parent, Quirarte, et al., 1995). Also, a rat's reaction to the shock in the shock-probe apparatus is much less intense than its reaction to a comparable shock in standard instrumental fear conditioning experiments (Treit & Parent, 1999), which suggests that the shock is less

intense in shock-probe. In addition, the shock used in the present experiment was escapable. In Pavlovian experiments, the shock is inescapable. For equal amounts of shock, inescapable shock appears to condition more fear than does escapable shock (Desiderato & Newman, 1971; Mineka, Cook, & Miller, 1984). Finally, one would expect that if the effects were due to overtraining, then the lesioned rats that received the most shocks during acquisition would have the best retention performance. However, there was no relationship between the number of shocks taken on the acquisition and retention tests in lesioned rats ($r = .201$; $p = .554$).

The ability of lesioned rats to effectively express memory on the retention test is also not likely due to a facilitatory, non-associative sensitizing effect of the preoperative shock experience. One characteristic of shock sensitization is that it appears to be context-independent (Davis, 1989). However, we have found that for both sham and amygdala-lesioned rats, the effect of preoperative shock experience on postoperative shock-probe avoidance is context-dependent. Specifically, unlike the present findings, the postoperative shock-probe avoidance of amygdala-lesioned rats given preoperative shock experience in a standard one-trial inhibitory avoidance paradigm is not different from that of shock-naive rats (Lehmann, Treit, & Parent, 1999). Thus, the most feasible interpretation of the present findings is that amygdala-lesioned animals remembered their previous shock experience and that the shock-probe avoidance paradigm permits the expression of the memory. Although this paradigm is typically used in studies of unlearned fear, the findings indicate that shock-probe is also an effective tool for assessing memory. In addition to allowing

rats to move freely while avoiding the probe, this task also has the benefit of providing more than one measure of memory.

Although amygdala lesions tended to affect the amount of time the rats remained immobile on the retention test, the effect was not significant. Immobility is an inverse measure of general activity levels, and previous findings have indicated that amygdala lesions increase activity levels (Burns et al., 1996; Lorenzini et al., 1991; Parent, Avila, et al., 1995; Parent et al., 1992; Vazdarjanova & McGaugh, 1998), although there are instances where no effect has been found (Maren, 1998; Treit et, al., 1993). It is not clear why there are conflicting results or why the effect on immobility was not significant in the present experiment.

The retention latencies of shock-experienced amygdala-lesioned rats tended to be shorter than those of shock-experienced sham-lesioned rats. This tendency does not likely reflect a memory deficit because spared memory was clearly observed in the lesioned rats when the number of shocks was used as the index of memory. This difference between the two measures suggests that the tendency toward shorter latencies in lesioned rats may reflect the effect of amygdala lesions on other processes that influence performance, such as activity levels. Indeed, it is interesting to note that the tendency for lesioned rats to have shorter retention latencies is paralleled by their tendency to have decreased immobility on the retention test. Regardless of the processes that influence the latency measure, it is clear that the retention performance of sham and amygdala-lesioned rats does not differ when the number of shocks is used as an index of memory.

Although it is not clear to what degree the shock-probe avoidance task involves Pavlovian and instrumental conditioning, it likely involves both. To effectively avoid the probe, the rat may need to form a Pavlovian association between the sight of the probe and the sensation of the shock. This would then be followed by the formation of an instrumental association between the sight of the probe and avoidance responses. The present findings indicate that rats with large amygdala lesions are not impaired in the ability to express the instrumental association. Evidence suggests that the Pavlovian association is also likely spared. Although naïve amygdala-lesioned rats receive more contact-induced shocks in the shock-probe test, lesioned rats selectively bury the electrified probe (Kopchia, Altman, Commissaris, 1992; Roozendaal, Koolhaas, & Bohus, 1991; Treit et al., 1993). This burying is directed specifically at the probe and suggests that amygdala-lesioned rats are able to make the association between the shock and the probe. Moreover, recent findings indicate that rats with amygdala basolateral complex lesions are able to express the memory of Pavlovian fear conditioning using instrumental responses (Vazdarjanova & McGaugh, 1998). Combined with our results, these findings indicate that, whether rats are trained in an instrumental or in a Pavlovian conditioning paradigm, amygdala-lesioned rats remember the cues associated with shock.

Kilcross, Robbins, and Everitt (1997) recently examined Pavlovian and instrumental conditioning simultaneously in the same rats. They found that lesions of the central nucleus of the amygdala disrupted Pavlovian conditioning but did not affect instrumental conditioning. The opposite effect was observed with lesions of

the basolateral region of the amygdala. Basolateral lesions disrupted instrumental conditioning but did not affect Pavlovian conditioning. They also found that large lesions that encompassed both regions impaired both types of conditioning. If one assumes that shock-probe conditioning involves both Pavlovian and instrumental fear conditioning, then one might expect that the large lesions induced in the present study should have impaired shock-probe avoidance. Although there are several procedural differences that could account for this apparent discrepancy (e.g., degree of training, food deprivation, apparatus), the findings are not actually incongruent if the timing of the lesion is taken into account. Although shock-probe avoidance is spared in rats given posttraining amygdala lesions (i.e., shock-experienced), our results indicate that shock-probe avoidance *is* impaired in rats given pretraining lesions (i.e., shock-naïve). Consequently, the finding that large lesions induced prior to training impair both Pavlovian and instrumental conditioning (Kilcross et al., 1997) is consistent with our results indicating that large lesions induced before training impair shock-probe avoidance.

The present results do not reveal the mechanisms underlying the deficit in shock probe avoidance that was observed in shock-naive rats. Our finding that the shock reactivity of sham and amygdala-lesioned rats did not differ suggests that this impairment is not due to decreased shock sensitivity. The possibility that the shock-naïve lesioned rats were not capable of learning the association between the sight of the probe and the sensation of the shock on the second shock-probe test is unlikely given the finding that amygdala-lesioned rats are still able to selectively bury the probe in response to shock (Kopchia et al., 1992; Roozendaal et al., 1991; Treit et al.,

1993). A remaining possibility is that amygdala-lesioned rats are unable to consolidate this association. That is, the amygdala may be involved in the transformation of a recent fearful memory into a long-term memory, and a deficit in this process would result in impaired shock-avoidance in shock-naïve amygdala-lesioned rats on the second shock-probe test. This possibility is supported by extensive findings indicating that the amygdala is temporarily involved in memory processes (McGaugh, 1989). For example, reversible inactivation of the amygdala shortly after training impairs subsequent retention performance; however, inactivation that is delayed by 6 or 24 hr has no effect (Bucherelli et al., 1992; Parent & McGaugh, 1994).

In conclusion, we show that rats given shock experience before the induction of large amygdala lesions are able to express memory of the pre-lesion shock experience. These findings indicate that the amygdala is not critically involved in the retention and expression of this, and perhaps other, learned fear associations.

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